Molecular & Biochemical Parasitology 164 (2009) 165–169

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Short communication

# Effects of DNA damage induced by UV irradiation on gene expression in the protozoan parasite *Entamoeba histolytica*

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ARTICLE INFO

*Article history:*
Received 17 April 2008
Received in revised form 3 December 2008
Accepted 10 December 2008
Available online 24 December 2008

*Keywords:*
*Entamoeba histolytica*
Amoebiasis
DNA damage repair
Double strand breaks
cDNA microarrays

ABSTRACT

Previously, we provided evidence for the role of *E. histolytica* RAD52 epistasis group genes and the EhRAD51 recombinase in DNA damage response. To identify other genes participating in DNA repair in this protozoan parasite, here we analyzed the transcriptional response to genetic damage induced by ultraviolet light (UV) using cDNA microarrays. We found that 11.6% (350 ORFs) and 17.2% (522 ORFs) of genes were modulated at 5 min and 3 h after UV irradiation, respectively. Most genes were less than 2-fold changed evidencing a weak transcriptional activation. The genes encoding so-called "classical" DNA repair proteins were slightly regulated in trophozoites submitted to UV irradiation. We also observed the over-expression of genes encoding for Fe–S clusters-containing proteins, potentially involved in the stress adaptation in response to DNA damage. Several genes encoding cytoskeleton proteins were repressed suggesting that actin dynamics was impaired after UV irradiation. Our analysis highlights novel genes potentially involved in DNA damage response, and these data will contribute to further elucidation of mechanisms regulating genome integrity in this early branch protozoan.



*Entamoeba histolytica* is an ancient eukaryote causative of human amoebiasis, which has a world-wide distribution with higher morbidity and mortality prevalence in the developing world, affecting more than 50 million people each year [1]. Despite its importance for human health, little is known about how this parasite modulates its gene expression during host infection. The successful survival of *E. histolytica* during infection requires parasite adaptation to the human host and the establishment of a transcriptional pattern that can accommodate the increased demands for nutrients, energy and responses to stress addressed by the immune system. Subsequent parasite survival implies adaptation of the parasite to the inflammatory response and later to the humoral immune response the host engages.

To establish successful host colonization *E. histolytica* trophozoites must also resist oxidative stress by free radicals and drug treatments, which could potentially causes DNA damage and cell death. Several common anti-parasitic drugs produce genotoxic damage, and various parasites have developed molecular strategies to overcome DNA injure [2]. However, molecular mechanisms that safeguard genome integrity and allow cell survival after DNA damage are poorly understood in most protozoan parasites.

The complete genome sequence of *E. histolytica* has been published [3], providing new opportunities for the study of gene expression profiles in parasites subjected to diverse stimulus and conditions. Microarray technology and genomic data have been successfully applied in *E. histolytica* to measure changes in transcripts abundance associated with virulence [4–6], heat shock [7,8], conversion between cyst and trophozoites forms [9], and epigenetic control [10].

In this work we addressed the question of transcriptional changes in *E. histolytica* treated by ultraviolet light (UV) that is known to produce oxidized bases, single-strand breaks, and cyclobutane pyrimidine dimers, which subsequently cause DNA double strand breaks (DSB), the most dangerous injury of DNA [11]. In eukaryotic and prokaryotic cells, the cellular response to DNA DSB activates a complex network of proteins that transiently arrest cell cycle and enhance DNA repair mechanisms. In case of extreme genetic damage, cells are targeted to apoptosis [12]. Two distinct pathways have evolved to ensure DSB repair: (1) non-homologous end joining (NHEJ) mechanism that requires the MRE11-RAD50-XRS2/NSB1 (MRX/N) complex, the DNL4 ligase, YKU70-YKU80 proteins and the LIF1-NEJ1 complex and (2) homologous recombination (HR) mechanism that depends on genes of the RAD52 epistasis group including MRX/N, RAD51, RAD51B-C-

*Abbreviations:* UV, ultraviolet light; DSB, double strand breaks; HR, homologous recombination; NHEJ, non-homologous end joining; ORF, open reading frame.

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doi:10.1016/j.molbiopara.2008.12.005

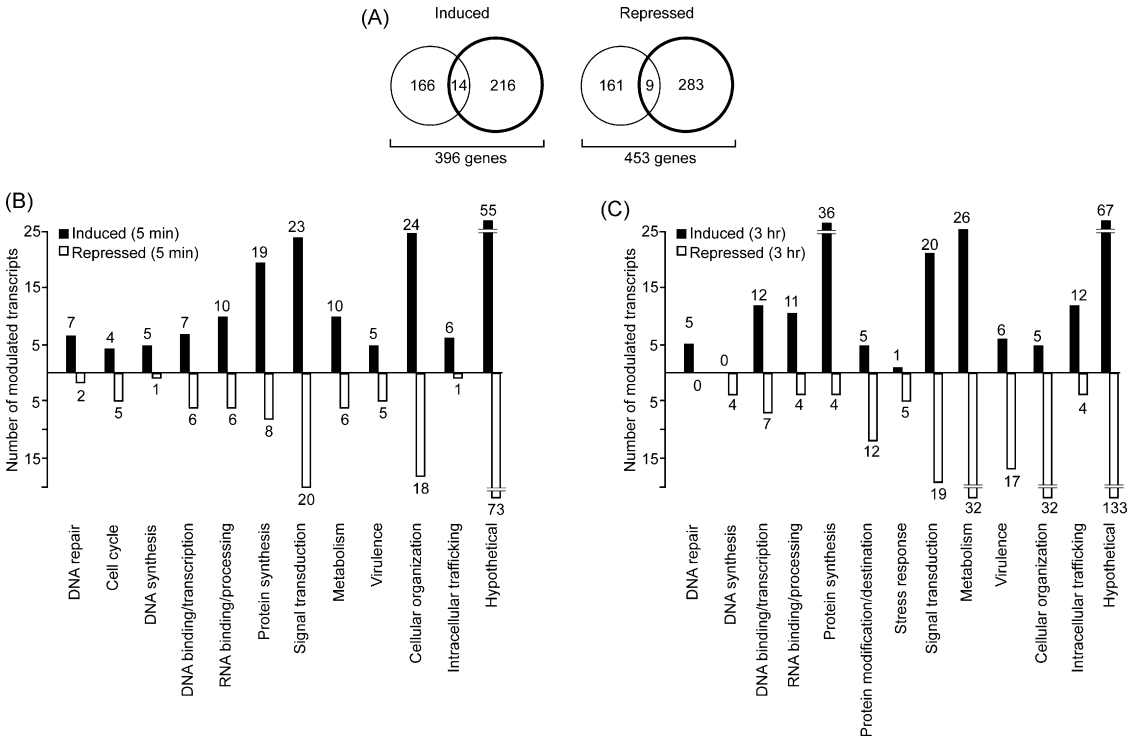


**Fig. 1.** Venn diagram of genes regulated after UV-C irradiation. (A) Overlap of genes regulated at 5 min and 3 h after UV-C irradiation is shown. Transcripts were included on the basis of a significant modulation ($P = 0.005$). Thin lines, genes changed at 5 min after UV-C irradiation. Bold lines, genes regulated at 3 h after UV-C irradiation. (B and C) Distribution of modulated genes into functional categories. The changed transcripts were assigned to a putative function on the basis of gene annotation in Pathema database and Pfam/COG families. The functional group is shown on the *x*-axis, and number of genes modulated in either a positive or negative way is indicated on the *y*-axis. (B) Transcripts induced at 5 min after UV-C irradiation are shown as solid bars and repressed genes are shown as white bars. (C) Transcripts induced at 3 h after UV-C irradiation are shown as solid bars and repressed genes are shown as white bars. In both B and C, the number of modulated transcripts is shown below each column.

D, XRCC2-3, RAD52, RAD54, RAD54B and RAD59, and additional genes represented by RFA1-3 which encodes the replication protein A (RPA), BLM helicase, DNA ligases and polymerases. HR also constitutes an accurate mechanism to recombine genetic information and generate diversity. Proteins participating in HR have been described in human and yeast cells and their role in DNA DSB repair by HR have been well documented [13].

Previously, we provided experimental evidence for the potential role of *E. histolytica* RAD52 epistasis group genes and the EhRAD51 recombinase in DNA repair [14]. To identify other genes participating in DNA damage response, here we extended our studies concerning DNA repair mechanisms and analyzed the *E. histolytica* global transcriptional response to genetic damage by cDNA microarrays. We used 70-mer oligonucleotides microarrays, which carry information of 3000 unique genes. Most of these genes were obtained directly from sequence analysis of *E. histolytica* transcripts. In addition, we have included almost all annotated genes potentially involved in DNA repair and described until today in Pathema database (http://pathema.jcvi.org/cgi-bin/Entamoeba/PathemaHomePage.cgi) or inferred from our direct computational genomic analysis [14]. *E. histolytica* HM1:IMSS strain trophozoites were irradiated with 254 nm UV-C light (150 J/m$^2$) for 8 s, and incubated in fresh TYI-S-33 culture medium at 37 °C for 5 min and 3 h after genotoxic treatment. Induction of DNA DSB in this DNA damage model has been previously corroborated by evaluation of EhH2AX histone phosphorylation status, TUNEL and comet assays [14]. Total RNA from non-irradiated and UV-C irradiated trophozoites was obtained by Trizol, retrotranscribed and labeled with Cy3 and Cy5 dyes; then microarrays were hybridized, washed and dried as previously described [7]. Four independent biological replicates were carried out in the experiment; two additional technical replicates were performed. Control trophozoites without UV-C irradiation were submitted to the same manipulation as treated cells. Microarrays data were analyzed as described [7]. Briefly, for normalization and differential analysis, scripts written with R software (http://www.R-project.org) were used. Lowess normalization was performed on all spots *Entamoeba* probes on a slide-by-slide basis (http://www.bioconductor.org). After pooling data from the technical and biological replicates (eight slides in total), a differential analysis was carried out. The Student test with Varmixt package was used and raw *P* values were adjusted by the Benjamini and Yekutieli method, which controls the false discovery rate (FDR). We considered genes with a Benjamini/Yekutieli value <0.05 as being differentially expressed at 5 min and 3 h after UV-C irradiation with respect to non-irradiated cells. An expression threshold, i.e., the mean of empty spots intensity + 2 S.D., was calculated on each slide. Complete experimental details and results are available online at http://genoscript.pasteur.fr.

The overall expression analysis identified *E. histolytica* genes that may contribute to adaptation and survival of trophozoites in response to DNA damage. We found that 11.6% (350 ORFs) and 17.2% (522 ORFs) genes were modulated at 5 min and 3 h after UV-C irradiation, respectively. A set of 180 genes were induced and 170 genes were repressed at 5 min, whereas at 3 h, 230 ORFs were up-regulated and 292 ORFs were down-regulated (Fig. 1A). Most genes selected from the strict statistical analysis performed here, were <2-fold modulated at both times, evidencing a weak transcriptional activation after DNA damage in *E. histolytica*. Similarly, it has been reported that human cells submitted to UV and ionizing irradiation showed a global transcriptional activation lower than 2-fold [15]. The overall set of expressed genes was clustered by Gene Ontology categories, which makes possible the identification of both hypothetical and characterized *E. histolytica* genes that may be regulated in response to UV-C irradiation (Fig. 1B and C). Many

**Table 1A**
*Entamoeba histolytica* up-regulated genes after UV-C irradiation.

| Oligonucleotide ID | Genbank accession number[a] | Gene product description | FC | *P*-value |
|---|---|---|---|---|
| 5 min after UV-C irradiation | | | | |
| EH-IP0778 | XM_643152 | Hypothetical protein (divergent RecA domain) | 2.067 | 3.6e−04 |
| 3 h after UV-C irradiation | | | | |
| Transcription and RNA processing | | | | |
| EH-IP1664 | XM_650702 | DEAD/DEAH box RNA helicase | 2.677 | 6.19e−12 |
| EH-IP0319.2 | XM_652250 | DEAD/DEAH box RNA helicase | 2.659 | 9.30e−12 |
| EH-IP2582 | XM_651763 | RNA polymerase subunit Rpb8 | 2.106 | 1.31e−08 |
| Protein synthesis and destination | | | | |
| EH-IP2596 | XM_648348 | Eukaryotic translation initiation factor 6 | 5.990 | 0.00e+00 |
| EH-IP0962 | XM_648252 | PCI domain. 26S proteasome | 2.022 | 1.18e−06 |
| Metabolism | | | | |
| EH-IP0581.2 | XM_650165 | Cisteine desulfurase. aminotransferase | 2.865 | 0.00e+00 |
| Oxidative stress protection | | | | |
| EH-IP0144 | XM_647032 | Iron-sulfur flavoprotein | 2.498 | 9.30e−12 |
| EH-IP0354.2 | XM_650796 | Fe–S cluster assembly protein NifU | 2.090 | 1.07e−07 |
| EH-IP0146 | XM_647039 | Rubrerythrin | 2.069 | 7.78e−07 |
| Miscellaneous | | | | |
| EH-IP1802 | XM_646999 | Hypothetical membrane-spanning protein | 4.553 | 0.00e+00 |
| EH-IP1181 | XM_652401 | BspA-like leucine rich repeat protein | 3.080 | 2.89e−13 |
| EH-IP0551.1 | XM_650260 | Glutamate-rich WD-repeat protein | 3.065 | 0.00e+00 |
| EH-IP1804 | XM_644643 | Protein kinase. putative CXXC rich | 2.845 | 0.00e+00 |
| EH-IP0816.2 | XM_649244 | Gal/GalNAc lectin light subunit 3 | 2.094 | 0.00e+00 |
| Unclassified | | | | |
| EH-IP1504 | XM_646115 | Hypothetical protein | 2.497 | 4.51e−11 |
| EH-IP1541 | XM_645887 | Conserved hypothetical protein | 2.393 | 8.61e−10 |
| EH-IP0704 | XM_649738 | Hypothetical protein | 2.078 | 4.60e−07 |
| EH-IP0246 | XM_646161 | Hypothetical protein | 2.060 | 5.47e−08 |

FC; fold change. Only genes with significant *P* values (0.005) are showed.
[a] http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi?term=ENTAMOEBA.

regulated genes were involved in potential DNA damage response pathways including cell cycle, signal transduction, and oxidative stress response. However, the majorities of predicted genes were involved in unexpected pathways including RNA binding and processing, cell structure, protein synthesis and degradation, energy metabolism, adhesion, vesicle trafficking, and a great number of hypothetical genes (Fig. 1B and C). Intriguingly, most of these genes have not been previously associated with the DNA damage response in other organisms.

## 1. Deep overview of genes regulated at 5 min after UV irradiation

To identify genes involved in the early response to DNA damage, we focused on changes in mRNA expression at 5 min after UV-C treatment (Table 1A). Results showed that only one gene (XM_643152) was 2.06-fold induced at 5 min after genotoxic stimulus. This gene encodes a hypothetical protein presenting no significant homology with any sequence in all databases and whose function is unknown. A detailed amino acids sequence analysis denotes that it has a divergent RecA recombinase specific domain. RecA/RAD51 family proteins catalyze homologous DNA pairing and strand-invasion reactions during recombination process [13]. In addition, several potential DNA repair genes presented slight transcriptional activation with significant *P*-value (0.005) (Table 2). These includes the putative *EhMre11* (XM_651393) and *EhRad50* (XM_647783) genes, whose homologous products function as early sensors of DNA DSBs in other organisms [16], as well as *EhRad23* (XM_644420), *EhDdb1* (XM_643241), and *EhRad54* (XM_648260) putative DNA repair genes (Table 2). On the other hand, results showed that three ORFs were 2-fold repressed at 5 min after genotoxic damage, including two hypothetical (XM_646498, XM_651305) and a BspA-like (XM_644548) genes (Table 1B). These data confirm that UV-C irradiation has subtle effects on gene transcription in cultured parasites at 5 min after genotoxic treatment, despite its ability to efficiently produce DNA damage [14]. Thus, it seems that the identification of proteins involved in early DNA damage response can be barely inferred from gene expression profiling in *E. histolytica* parasite.

## 2. Deep overview of genes regulated at 3 h after UV irradiation

In contrast to the weak transcriptional response at 5 min after genotoxic injure, the effects of UV-C irradiation on *E. histolytica* gene expression were more evident at 3 h after treatment. Gene expression profiling showed that 18 genes were >2-fold induced at 3 h after UV-C irradiation (Table 1A). A putative elF6 gene was 5.99-fold up-regulated. Notably, we also detected genes which potentially encode proteins involved in mRNA processing, including two putative RNA helicases (XM_652250, XM_650702) of the DEAD/DExH box family recently described in *E. histolytica* [17], which are involved in mRNA splicing and translation in other organisms. In addition, an ORF (XM_651763) which predicts a protein that shares 31% identity with human RBP8 subunit of RNA polymerase II exhibited a 2.1-fold change at 3 h after UV-C irradiation. It has been reported that DEAD-box RNA helicases, which catalyze the unwinding of duplex RNA secondary structures, play essential roles in DNA repair, recombination, transcription, and mRNA translation [18]. Recently, it has been reported that human BRCA1 DNA repair protein regulates RPB8 in response to DNA damage, inducing a transiently inhibition of 3′-end mRNA processing to allow transcription-coupled DNA repair and cell survival [19]. However, DEAD/DExH box RNA helicases and RBP8 functions after DNA damage remain to be elucidated in *E. histolytica*. Remarkably, UV-C irradiation induces oxidation of DNA bases and genotoxic stress. Here, we detected the up-regulation of three ORFs (XM_647032, XM_647039, XM_650796) codifying

**Table 1B**
*Entamoeba histolytica* representative down-regulated genes after UV-C irradiation.

| Oligonucleotide ID | Genbank accession number[a] | Gene product description | FC | *P*-value |
|---|---|---|---|---|
| 5 min after UV-C irradiation | | | | |
| EH-IP1823 | XM_644548 | BspA-like leucine rich repeat protein | −2.127 | 6.28e−05 |
| EH-IP1434 | XM_646498 | hypothetical protein | −2.024 | 2.38e−04 |
| EH-IP1323 | XM_651305 | hypothetical protein | −2.000 | 7.02e−04 |
| 3 h after UV-C irradiation | | | | |
| Cytoskeleton organization and lysine rich proteins | | | | |
| EH-IP0920.1 | XM_648537 | KERP1 | −3.460 | 0.00e+00 |
| EH-IP0472 | XM_644616 | Actobindin homolog | −3.333 | 0.00e+00 |
| EH-IP1273 | XM_647376 | Actin | −2.923 | 5.37e−13 |
| EH-IP0477.2 | XM_650398 | Actin | −2.747 | 1.45e−11 |
| EH-IP0821.3 | XM_649180 | KRiP3 | −2.518 | 5.87e−10 |
| EH-IP0660 | XM_643674 | Rho family GTPase | −2.475 | 2.22e−09 |
| EH-IP0764.3 | XM_651936 | Myosin II heavy chain | −2.433 | 2.41e−10 |
| EH-IP0353.3 | XM_650801 | KRiP2 | −2.421 | 2.28e−10 |
| EH-IP0837 | XM_651826 | LIM domain protein | −2.352 | 3.47e−10 |
| EH-IP0116.2 | XM_647235 | Actin binding protein | −2.336 | 2.22e−09 |
| EH-IP0287 | XM_645834 | Coactosin, actin depolymerisation factor | −2.304 | 1.06e−09 |
| EH-IP0217.1 | XM_646433 | Erythrocyte binding protein | −2.267 | 2.17e−08 |
| EH-IP0451.2 | XM_644794 | α-Actinin 2 | −2.000 | 3.20e−06 |
| Energy metabolism | | | | |
| EH-IP1001.1 | XM_647931 | Alcohol dehydrogenase 3 | −2.564 | 1.12e−10 |
| EH-IP1009 | XM_647892 | Pyruvate:ferredoxin oxidoreductase | −2.463 | 8.68e−10 |
| EH-IP0758.1 | XM_643193 | Truncated pyridine nucleotide transhydrogenase | −2.262 | 6.34e−10 |
| EH-IP1913 | XM_644148 | Alcohol dehydrogenase 2 | −2.207 | 4.63e−07 |
| EH-IP1387 | XM_646746 | Transketolase | −2.132 | 1.80e−07 |
| EH-IP0743.1 | XM_649574 | Pyruvate phosphate dikinase | −2.049 | 8.17e−06 |
| Miscellaneous | | | | |
| EH-IP1620 | XM_645626 | Methionine gamma-lyase | −2.785 | 7.52e−13 |
| EH-IP0409 | XM_645064 | Cysteine protease 1 | −2.309 | 1.04e−08 |
| EH-IP0796.3 | XM_649355 | Gal/GalNAc lectin subunit Lgl1 | −2.288 | 9.20e−08 |
| EH-IP1881 | XM_644363 | Reverse transcriptase | −2.217 | 8.06e−08 |
| EH-IP0992 | XM_648070 | Heat shock protein 90 | −2.109 | 2.06e−06 |
| Unclassified | | | | |
| EH-IP0943 | XM_651628 | Hypothetical protein | −5.586 | 0.00e+00 |
| EH-IP0264 | XM_646068 | Hypothetical protein | −4.310 | 0.00e+00 |

FC; fold change. Only genes with significant *P* values (0.005) are showed.
[a] http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi?term=ENTAMOEBA.

for iron–sulfur clusters-containing proteins, potentially involved in oxidative stress protection (Table 1A). Interestingly, a role for Fe–S clusters as cofactors in DNA repair has been proposed [20]. Moreover, some enzymes involved in DNA recognition and repair carried Fe–S clusters. Other genes potentially involved in DNA repair were also detected as up-regulated 3 h after DNA damage ($P = 0.005$), such as the putative DNA mismatch repair *EhmutS*, *EhRad52*, *EhRad24* and mutt/nudix family genes, although they exhibited a weak transcriptional activation (Table 2). The analysis of down regulated genes showed that 26 genes were 2-fold repressed at 3 h after UV-C treatment (Table 1B). Of these, two genes (XM_651628, XM_646068) encoding hypothetical proteins were severely repressed. Interestingly, 12 down-regulated genes correspond to cytoskeleton and associated factors. These include actins (XM_647376, XM_650398), actin binding proteins (XM_644616, XM_647235), coactosin (XM_645834), and myosin II (XM_651936) proteins. We suggested that down-regulation of these genes may contribute to slow down the dynamics of amoebic cytoskeleton, which could influence proper cell cycle progression after DNA damage in trophozoites. However, additional experiments are needed to corroborate this hypothesis. Three genes encoding lysine rich proteins from the KRiP family (KERP1, KRiP2 and KRiP3) involved in amoebic pathogenesis [6] were also down-regulated, giving a new potential insight for these factors in the response to UV-C stress in

**Table 2**
*Entamoeba histolytica* putative DNA repair genes up-regulated after UV-C irradiation.

| Oligonucleotide ID | Genbank accession number[a] | Gene product description | Functional category | At 5 min | | At 3 h | |
|---|---|---|---|---|---|---|---|
| | | | | FC | *P*-value | FC | *P*-value |
| EH-IP2359 | XM_648260 | DNA repair protein RAD54 | DNA repair | 1.72 | 4.92e−04 | | |
| EH-IP0637 | XM_649978 | Phosphatidylinositol-4,5-bisphosphate 3-kinase | DNA repair/signaling | 1.33 | 1.18e−02 | | |
| EH-IP1231 | XM_651393 | MRE11 double-strand break repair protein | DNA repair | 1.29 | 4.44e−02 | | |
| EH-IP0499 | XM_644420 | RAD23 protein | DNA repair | 1.26 | 3.49e−02 | | |
| EH-IP2545 | XM_643241 | Damaged DNA binding protein DDB1 | DNA repair | 1.25 | 4.95e−02 | | |
| EH-IP0039.1 | XM_647783 | DNA repair protein Rad50 | DNA repair | 1.23 | 3.94e−02 | | |
| EH-IP1545 | XM_647442 | DNA mismatch repair protein mutS | DNA repair | | | 1.55 | 1.21e−03 |
| EH-IP1013.2 | XM_647854 | 14-3-3 protein 1 (RAD24) | DNA repair/signaling | | | 1.51 | 1.24e−05 |
| EH-IP1253 | XM_651492 | mutT/nudix family protein | DNA repair | | | 1.48 | 8.37e−03 |
| EH-IP1478 | XM_651011 | Rad52/22 family double-strand break repair | DNA repair | | | 1.43 | 8.37e−03 |

FC; fold change. Only genes with significant *P* values (0.005) are showed.
[a] http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi?term=ENTAMOEBA.

*E. histolytica*. To validate the changes in mRNA expression detected by the microarrays assays, we performed qRT-PCR for representative up- and down-regulated genes. Results showed an acceptable correlation between microarray and qRT-PCR data for the four analyzed genes (Supplementary table S1).

This work represents the first genome-wide analysis of DNA damage response induced by UV-C irradiation and its effects on gene expression in *E. histolytica*. The first important finding from our experiments was that genes encoding so-called "classical" DNA repair proteins were slightly modulated in trophozoites submitted to UV-C irradiation. This was not surprising given that similar results in yeast and mammals showed no-relationship between genes whose expression is increased after different DNA-damaging treatments [15]. These data suggest that trophozoites have enough basal levels of conserved DNA repair proteins and most regulation in response to UV-C irradiation could be operating at post-translational level in *E. histolytica*. Secondly, we observed a stress response to UV-C treatment represented by the over-expression of several genes encoding Fe–S clusters-containing proteins potentially involved in the stress adaptation in response to DNA damage. The third finding was that genes encoding several cytoskeleton proteins were notably repressed suggesting that *E. histolytica* actin dynamics was impaired after UV-C irradiation to permit proper DNA damage repair. Finally, our analysis highlights novel genes potentially involved in DNA damage response in this parasite, which are currently used as a guide for experimental studies. These insights reveal new directions for studying the response to DNA damage, and they will contribute to the further elucidation of mechanisms regulating genome integrity and variability in this early branch protozoan.

## Acknowledgments

We thank Jean Yves Copee, Odile Sismeiro and Ghislaine Guigon for their help with microarray analysis. We also thank Sollange Archer for artwork assistance. This work was supported by a grant from the Pasteur-Weizmann Research Council and Mexican grants from CONACyT, UACM, COFFA-IPN, and SIP-IPN. Cesar Lopez-Camarillo was recipient of a fellowship from the French Embassy in Mexico.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molbiopara.2008.12.005.

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